Journal of Research on the Lepidoptera 10(3):203-214, 1972

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NOTES ON DESCRIBING, MEASURING, PRESERVING AND PHOTOGRAPHING THE EGGS OF LEPIDOPTERA

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Note: Any figure numbers mentioned in this paper refer to egg photographs in the paper that follows (p. 215).

Describing Eggs

In the study of (any) lepidopterous eggs, some important differences to look for, and always worth recording in the notebook, are listed below:

(1) Details on the *chosen site(s) for oviposition* under natural conditions. If this information is not available, it is often possible to speculate on it, often with a fair degree of accuracy, by observing where and how a captive female places her eggs during confinement. What type of surface appears to be most acceptable—smooth or rough? Are the eggs deposited exposed, in the open, or in crevices or otherwise hidden locations? What type of ovipositor does the female have?

(2) *The mode or pattern of oviposition.* In captivity, this often provides further clues concerning the above. The mode of oviposition is often (not always) consistent among related species, but can show wide variation at the subgeneric or generic level. Are the eggs dropped free (in no way adhering to anything), or are they attached to a substrate? If attached, note color (if any) and strength of the adhesive (securely vs. weakly-glued). In what pattern and alignment are they deposited: Singly; in two's and three's; in short or long, curving or straight rows (end-to-end or side-to-side); in flat (single-, double-, or multiple-layer) masses, or heaped-up masses; in piles or clusters; in stacks; or in any other formations, which may be regular or ir-

regular? Are they deposited end-up, or on their sides? Is each egg touching or overlapping the next, or are all distinctly separated? If touching, are they also *adhering* to each other as well? All aligned in the same direction, or variable in alignment?

(3) *The covering, if any.* Naked versus coated (or partially-coated) with deciduous, hair-like (or fluffy) scales from the end of the female's abdomen, or with some other substance (*i.e.,* a dried frothy or foam-like covering; soil particles, etc.).

(4) *The basic shape* or outline of the egg, and its various profiles when viewed from different positions.

(5) *The relative hardness,* softness, or flexibility of the chorion (= shell). This can vary tremendously.

(6) The type (and extent) of *surface sculpturing* on the shell, such as ribs, grooves, pits, granulations, or pustules, etc.

(7) *Degree of luster* or surface-shine (gloss or sheen); some (relatively few) eggs show little or no luster. Whatever the case, this is an important feature.

(8) *Transparent* and/or *translucent* versus completely *opaque* shells.

(9) *ALL color changes* that are observed during the incubation period, and any variations noted in these color changes. Exceptions: If the shell is completely opaque, no color change will be seen (as in many anthelids, saturniids, or lasiocampids, and in some notodontids and thaumetopoeids, etc.).

(10) *Measurements* of all dimensions should be recorded. These are valuable for comparison with the egg dimensions of related species already known.

(11) A statement as to whether the egg appears *large or small for the size of the adult female.* The decision as to whether a species deposits a "large" or "small" egg cannot always be made, but it is very often instantly apparent, provided that the observer has already had a fair range of experience with lepidopterous eggs. The reasons I consider this concept to be of value are discussed in the fourth paragraph of the second paper following this one.

(12) The *hour of larval emergence,* and through which region of the egg (side, top, or end)? Is the exit-hole clean-cut and perfect, or rough and irregular?

(13) After larval emergence from the egg, is the *shell devoured* —entirely, partially, or not at all?

When comparing lepidopterous eggs, it is important to consider *all* of the above points—not just the surface morphology, color changes, and measurements. By recording all of these points (plus any others that family or generic specialization may disclose to be important), it is frequently possible to glean some very nice separations for closely-related species, without obligatory reference to micropylar detail or S.E.M. microphotography. Knowledge of this fact could be of considerable moral support to isolated field workers or amateurs, possessing only a hand lens or modest microscope, but having, nevertheless, an abundance of enthusiasm and sharp powers of observation. (See Wheeler, 1939). Some workers in this category have much to offer, but will eventually die, publishing nothing, for fear of not "measuring up" to the more sophisticated productions of present day professional entomology. These losses are *everyone's.* In some instances the losses will be irreversible, as more and more unique habitats are destroyed (worldwide), and the *few* who really knew these localities die with them—*publishing nothing.*

Measuring and Comparing Eggs

A convenient and consistent method for expressing, recording, and comparing the measurements of "Macro" eggs is presented here. Refinements are included by means of which it is possible to express all observed variations in the egg dimensions of a single species, and to infer *which* (of all measurements shown) is the most typical noted for each dimension (based on the series measured). I have evolved this method over the past 5 years, while rearing about 180 "Macro" species from the egg in South Australia, and feel that it could now be presented for consideration by others doing similar work, who might find in it a consistent and workable system. It is *not* recommended as a method for measuring the eggs of smaller "Micros", but is very satisfactory for most "Macro" eggs. Finer measurements and equipment are needed for the former.

This system can be employed by field workers anywhere, requiring only one precision instrument—*metric dial calipers, accurate to 0.05 mm.* (1/20 mm.). The Japanese "Peacock" brand is one of several available in Australia (price was approx. $24.00 in 1967). A good hand lens, used to make certain of accurate positioning of the egg during all measurements, is a necessity, at least for the smaller "Macro" eggs. The reading can be considered correct when the calipers are *just* able to pick up (and hold) the egg securely, but *without* denting its

shell. (The minutest release-turn of the dial should drop the egg.) Avoidance of dried adhesive, or other foreign material on the surface, must be kept in mind when measuring eggs that were heavily-glued or scale-coated, etc. An attempt should always be made to obtain and measure a *series* of eggs from one or more females, watching for variation in size or shape.

Metric dial calipers are available in various brands and calibrations, some calibrated finer than 0.05 mm., but smaller measurements do not appear to be required (or even desirable) among *most* of the "Macro" eggs I have studied to date. Measurements finer than 0.05 mm. can become quite meaningless, and often serve only to cloud or needlessly complicate the picture, due to the considerable variation in size which can be encountered among the eggs from a single female.

Size variation, among the eggs from a *single* female, *commonly* falls in the range of 0.05 to 0.10. The eggs of a larger South Australian oenochromine geometrid, *Monoctenia falernaria* Gn., have been observed to vary by as much as (up to) 0.55 mm., in one of the dimensions (maximum length), within a series obtained from *one* female (Blackwood, S.A.)! (See Fig. 28 and commentary, in the paper that follows). Such considerable variation as this is apparent even to the unaided eye. Sometimes the shapes or proportions of eggs vary to a certain extent; this also applies in the case of *M. falernaria.* There is often a nice separation in egg size between some of the species in a genus; conversely, there can also be considerable or complete overlap at the extreme measurements recorded, especially when egg size variation is great within a species, as in the case of *M. falernaria.* (Compare the measurements of *M. smerintharia,* Fig. 27, with those of *M. falernaria;* those of the latter entirely encompass those of the less variable *M. smerintharia* egg).

After *M. falernaria,* the most variation in egg measurements (among South Australian moths reared to date), has been recorded for the following four species: Notodontidae—*Danima banksiae* Lew. (0.45 mm. maximum variation in diameter, between two widely-separated populations); Geometridae, Ennominae—*Thalainodes macfarlandi* Wilson (up to 0.30 mm. variation in length), *Cleora bitaeniaria* (Le Guill.) (0.20 mm. maximum variation in length); Anthelidae, Munychryiinae—*Munychryia senicula* Walker (0.20 mm. maximum variation in length). The complete measurements for the egg of *M. senicula* (from Highbury, South Australia) are: 1.40-*1.35*-*1.30*-1.20 x

1.10-*1.0*-0.95 x 0.95-*0.85*-0.80 mm. For photographs of the egg (and other stages) of *M. senicula,* see Common and McFarland (1970).

Egg shapes can be reflected (and thus automatically compared) by the manner, and the sequence, in which the measurements of their various dimensions are set down. (It seems advisable to do away with such terms as "length", "width", "height", or "diameter", which cannot always be rendered analogous). This system involves recording of *all* dimensions of the egg in a sequence of DIMINISHING MAXIMUMS. (See numerous examples in next paper). At the same time, it is possible to include the complete range of variation observed for each of the dimensions (based on a series measured); it can also be indicated which measurement, within each dimension, is the more usual or "normal", if this information is possible to glean from the available eggs.

In order to explain what is meant by "diminishing maximums", it is first necessary to briefly describe six basic egg shapes commonly encountered among "macro" moths: (1) Spherical eggs, having only *one* consistently measurable dimension; (2) nearly spherical eggs, having *two* slightly differing dimensions, such as those of the geometrids *Phallaria ophuisaria* Gn. (Fig. 29), *Idiodes apicata* Gn. (Fig. 31), or *Amelora leucaniata* Gn., etc.; (3) more-or-less hemispherical eggs, having *two* differing dimensions, as in many notodontoids and noctuoids (Figs. 10, 11, 12, 14, etc.); (4) cylindrical or subcylindrical eggs, having *two* very contrastingly different dimensions, as typified by the geometrids *Rhynchopsota rhyncophora* (Lower) (Fig. 30), *Stibaroma melanotoxa* Guest (Fig. 32), *Cleora bitaeniaria* (LeGuill.) (Fig. 33), and numerous other moths; (5) more-or-less losenge-shaped eggs, having *three* clearly measurable maximum dimensions, as typified by many of the Geometrinae (Fig. 23) and Ennominae; (6) eggs still having *three* clear-cut maximum dimensions, but *tapering* notably toward the smaller end, as typified by the ennomine geometrids *Mnesampela fucata* (Feld.) (Fig. 35), *Niceteria macrocosma* (Lower) (Fig. 40), *Stathmorrhopa macroptila* Turner, and some of the Geometrinae.

Taking, as an example, a series of spherical or near-spherical eggs, a number of them are measured, making a special effort to include all eggs appearing to be slightly larger or smaller than the majority, as well as whatever appear to be typical sizes and shapes in the available series. If any variation is found,

it can be expressed as follows: "Size = 1.30-*1.25*-1.20 mm.". This implies that the (maximum) diameter recorded, for the largest egg(s) in the series measured, was 1.30 mm.; that the (maximum) diameter recorded for the *smallest* egg(s) in the series measured, was 1.20 mm.; that 1.25 mm. (italicized) was the maximum diameter recorded for the *majority* of eggs in the series measured. If the variation in size was found to be about equally divided among the eggs measured, it would be recorded simply as: "Size = 1.30-1.20 mm.". (The insertion of the "*1.25*" is a refinement in interpretation, not worth including unless an adequate series has been measured, and the majority clearly fall between the two extremes). This italicized measurement (if included) should not always be interpreted as the average; see examples in Figs. 32, 33 and 38 of the paper that follows (last line under each commentary). In Fig. 33, a number of eggs measured in one series (Blackwood S.A.) varied from 1.25 down to 1.05 mm. in max. length, but by far the majority were found to have a max. length of 1.20 mm., or very close to it. Thus, *1.20* is italicized (or underlined) as the "typical" length for eggs of the Blackwood population of that species.

Taking eggs having two dimensions, Figs. 10-20 provide good examples. Looking at Fig. 12, the dimensions are 0.90-*0.85*-0.80 x *0.75*-0.70 mm., recorded in a sequence of diminishing maximums. The first set of measurements (preceding the "x") refers to the (maximum) diameter in this case, the most common diameter in the series measured being 0.85 mm., or close to it; the second set of measurements (following the "x") refers to the height in this case, the most common maximum height in the series measured being 0.75 mm. Looking at Fig. 13, the dimensions recorded are 1.25-1.20 x 1.00-0.85 mm. The first set of measurements (1.25-1.20) refers to the maximum length in this case, which was observed to vary by 0.05 mm. in the series measured; the second set refers to the diameter in this case, which is seen to vary to a greater extent (0.15 mm.) than the length. Comparing the (complete) measurements of Fig. 12 with those of Fig. 13, it can be seen that these represent two very different egg shapes; the corresponding photographs depict this obvious difference in proportions. Fig. 14 shows an egg with its two dimensions almost identical, but its diameter is usually a little greater than its height. Fig. 18 is an example showing little or no variation (to 0.05 mm.) in either of its two dimensions (thus, recorded simply as 0.70 x 0.60 mm.);

in this case minor variation might be expected to show at measurements *finer* than 0.05 mm. Yet, in contrast with many geometrid eggs, these of the arctiid, *Nyctemera,* are notably uniform in their dimensions.

Taking an example from eggs having three dimensions (length x width x height), Fig. 35 is chosen for discussion because of the fact that this egg tapers from thick at one end to much smaller at the other. When height, width, or diameter decline from one end of an egg to the other, it should be the *maximums* (of all dimensions) that are measured and recorded. (The tapering height of many geometrine eggs represents a less extreme example, often declining very slightly from one end to the other).

To summarize this system of diminishing (or decreasing) maximums: Variations in egg dimensions are uniformly recorded; all dimensions and measurements are arranged in a continuous sequence, from maximums of the largest to maximums of the smallest eggs in the series measured; all recorded measurements imply *only* the maximums for each egg measured in a given series; variations (in the maximums) of one dimension are separated from each other by hyphens; any second or third (new) dimension is separated by an "x" from any preceding it. Some reasons to recommend this system are as follows: It permits arrangement, of all recorded measurements for all dimensions, into a neat gradient series without any interruptions (or transpositions) in the flow. Where overlap between two dimensions occurs, this is instantly apparent, without the need to shuffle figures. In most cases a fairly accurate image of the egg proportions can be visualized while reading the measurements. (Before looking at the photos, compare the measurements for Figs. 7 and 8, trying to imagine how they will differ in shape). Any need for the inclusion of words (sometime ambiguous), such as "length", "width", "diameter", or "height", is eliminated. Both the recording and the comparing of egg dimensions are simplified, while increasing the amount of information conveyed.

A system of *increasing* maximums (equivalent to any of my measurements if recorded entirely in reverse, from right to left) could be used in the same way. However, the sequence of *diminishing* maximums seems to lend itself more naturally to the process of recording egg measurements.

Dry Preservation of Eggs and Larval Exuviae

Empty (hatched) egg shells (or fragments thereof) are well worth saving, if not too badly collapsed or devoured. They

are always valuable as comparative material in a life history collection, or for future study and photography. They show surface features better than alcoholic material, when held under the right type of lighting. There is never any swelling, as is sometimes the case with eggs in fluid preservatives. Accurate measurements can be obtained from empty shells, if they are not too flimsy, or partially eaten. The dry shells often convey information about the mode of attachment (singly or otherwise), location of larval exit-hole, whether or not the shells were partially consumed, and so on.

Empty egg shells are quite adaptable to certain photographic techniques, including S.E.M. (see Figs. 41-48 in the paper that follows); they may be preferable for the latter, because they are already dry, and (usually) relatively clean. If not sufficiently clean, they are quickly and easily cleaned in most cases. The shells can be stored in *small,* clean, dry glass tubes or vials, into which should be inserted labels tying them to the corresponding adults and larvae. The labels should fit tightly inside the tubes so that there is no label movement if the tube is shaken; this prevents damage to delicate or flimsy egg shells if the tubes are mailed or roughly handled. They may be left as attached to leaf pieces or bits of twig, if these are thoroughly dried prior to closing the tubes. If the eggs were attached to plastic bags, paper, or muslin, this is easily cut down to fit into the tubes. The tubes (and contents) must be thoroughly dry when closed, or mould will rapidly develop. Store in a cool, dark, *dry* place.

A very worthwhile addition to the dry eggshell collection (whenever numbers permit), is to kill a few of the young larvae while still in their eggs, *just before hatching,* by placing the eggs in a freezer for several days. This must be done *before* they have started chewing through the shells, the object being to obtain dry egg specimens with entirely perfect surfaces and no collapse. They should be as close to hatching as possible, which usually insures that no collapse of shells will take place after the larvae inside have been killed. (Controlled heating in an oven might also prove to be a successful technique). Dry egg specimens obtained by freeze-killing the larvae are superior to hatched shells because of the completely undamaged chorion and the increased rigidity, making them easier to handle during measurement or preparation for photography, etc. If only a few eggs are obtained from a confined female, it may not be advisable to kill any larvae still in the eggs, if one also hopes to pre-

serve specimens of all larval instars, pupae, and adults from the same eggs. It is in such cases that the hatched egg shells are most valuable to save, when otherwise no dry egg specimens would be preserved in *any* form. Unfortunately, the micropylar area is often partially damaged or completely destroyed during larval emergence from the egg, although the hatched shells of some species, such as the Australian ennomine geometrid, *Idiodes apicata* Gn., may show the micropyle intact (see Figs. 46-47 of the paper that follows).

It is often convenient to keep early instar exuviae in the same tube with the dry egg shells; they will not damage each other. If no larvae have been preserved in the first instar, these cast skins and head capsules become particularly important to save. Second instar head capsules and cast skins can also be enclosed in the same tubes with the dry egg shells. Exuviae of any subsequent (larger) instars, if saved dry, are better kept in other tubes separate from the egg shells.

Uncomplicated Egg Photography

In the introductory section of the paper that follows, the camera and microscope set-up used (for Figs. 1-40) is briefly described. Additional details are included below, to cover some of the techniques that were used to obtain these photographs.

Lighting: I have found bright morning sunlight preferable to electronic flash for egg photos of this nature, because it is easier to achieve an entirely predictable modelling (three-dimensional effect) with the sunlight. The eggs can be carefully scrutinized (through the camera which is attached to the microscope), and their positions can be minutely adjusted until exactly the desired effect is obtained, with reference to light and shadow on surface-sculpturing or pits, etc., so as to bring out any such details through the intentional (but moderate) use of shadow. Shadow should never be entirely eliminated from egg (or pupal) photos such as these. Flash tends to penetrate too uniformly and completely into small grooves and pits, etc., often flooding them out or rendering them more-or-less obscure. *Low-positioning* of the flash head, coupled with the use of reflectors ("bounce") can, of course, eliminate this to some degree. Yet, it is still sunlight that best allows one to study the photo at leisure, and then to photograph *exactly what was seen*, once all adjustments have been made to the photographer's satisfaction. Morning sun, coming in through an open (or very clean if closed) window, is almost always

quite suitable for this type of photography, *provided* the sky is brilliant and clear; under hazy, smoggy, or cloudy conditions, such lighting is not as suitable. Brilliant artificial light sources then become preferable.

In photos such as these, it is most important to capture surface shine at its full value; however brilliant it may be, it must not be eliminated or reduced. Shine or gloss on the chorion surface often varies greatly between the eggs of different species, and is therefore a most useful taxonomic aid to characterizing any lepidopterous egg. In the paper that follows, the relative amount of surface-shine is consistently reproduced for each egg depicted (appearing as highlights or variable white areas on the eggs); it can be seen to vary from brilliant surface shine (Figs. 9, 14, 24, and 25, etc.), to moderate or slight sheen (Figs. 7, 26, 30, and 40, etc.), to little or no sheen (Figs. 8, 10). Surface texture usually determines the degree of shine; generally, the smoother the surface the more shine.

Exposure-time for Figs. 1-40 were mostly in the range of 1/15 to 1/2 second, with the majority being 1/8 or 1/4-second exposures. B. & W. films of slower speeds were used: ASA 20, 32, and 50 (mostly ASA 32). Faster films are also excellent for such photos, if of very fine grain. The selection of film depends, to a large extent, on the type of lighting preferred and the results desired.

Backgrounds chosen are of utmost importance to the whole picture. If the eggs are removed from the substrate to which they are attached, they should first be carefully cleaned, by removing any adhering scales or other particles (dust, etc.). A series may then be placed on a selected background, and positioned so as to show all major profiles. Of materials available in Australia, I have found small squares of "Perspex" (acrylic sheet plastic, ± 1/8-inch thick) to be superb egg (or pupal) backgrounds. Perspex is available in numerous colors, and is mostly *translucent*, sometimes transparent. Being translucent, it "absorbs" shadows (greatly softens them), and the eggs may be set down directly upon the plastic surface. If static develops, causing the eggs to jump erratically about when being positioned, this trouble is easily overcome by rubbing the Perspex plate with an anti-static cloth before attempting to use it.

It is convenient to have on hand several colors of Perspex, cut into small plates (1-inch to 2-inch squares). The most useful colors to have are translucent milky-white ("Opal"

Perspex), translucent gray-white, translucent (but *not* transparent) pale blue and pale gray, water-clear (colorless and transparent), transparent deep purple, and jet black. It is also highly desirable to sand some of these small plates (on *one* side), with very fine Emory paper, using a gentle rotary motion. These sanded plates will have a matte finish (no shine), which is desirable in many photos, but the shadow-absorbing power of the Perspex will be somewhat reduced. The various clear plates can also be stacked upon one another to achieve subtle variations in background effect. With a set of small plates including *all* of the above colors (plus any others, as requirements dictate), good results can be obtained with eggs of almost any opacity or color. These small plates are of great value because they may be slowly turned, without disturbing the arrangement of eggs on them, in order to obtain the best positioning with relation to light source (for maximum surface definition, or highlights in shiny areas, etc.). If the eggs are left attached to the original substrate, the choice of background is usually less important. Shadows will often be darker and may cause trouble. Different lighting (source and/or direction, plus use of flash "bounce", etc.) can overcome these problems. For most egg photographs, it is desirable to remove at least a few of the eggs from the original substrate, unless one desires to first depict the mode of oviposition, undisturbed (Figs. 17, 18, and 19, for example); *both* types of photos have their values.

If moths can be induced to oviposit inside thin, colorless polyethylene bags, as earlier recommended by Peterson, the eggs are most easily handled and photographed. It was by this method that I obtained Fig. 4; it would not have been possible to remove them from the substrate without damage, so it was necessary that they be deposited on a flat and transparent surface in order to get photos from beneath, and also in order to be free to make use of whatever background might be required.

I have described the above techniques to demonstrate some ways that good results can be obtained in any location, often with the simplest of equipment. There should be no compromise with the quality of the camera, lenses, or microscope, however, which should be the *best* the worker can obtain. Beyond this, it is *not* necessary to have access to a laboratory full of elaborate, expensive equipment in order to produce good photographs; all can be done at home, on a good solid table, using various small props and gadgets made from wood, plastic, and scraps

of metal, etc. There are few limitations in this sphere, aside from the time available for the work, or the worker's imagination. (See Karp, 1966—a *most* valuable reference).

The more conventional insect egg photographs, at lower magnifications (less than 100x), as in Figs. 1-40 of the paper that follows, or after Peterson (1960-63), have great value as taxonomic aids. Yet, when photographs like these are compared with those taken by more recently-developed scanning electron microscope techniques (for example, Figs. 41-48 of the paper that follows), they are seen to be rather elemental!

My object here is not to dwell upon this undeniable fact; rather, to suggest that it is still of great importance for workers to *continue* producing (and publishing) egg photos at lower magnifications. The primary reason for this statement is that there always will be a need to bridge the huge gap which exists between the field (what is seen with the naked eye, or, at best, with a hand lens)—and the lab (what can be produced by S.E.M. techniques). Somewhere in between these two extremes, it is possible to compare or relate the photograph in *either* direction—back to what was seen with the naked eye (or a hand lens), OR up to what will be produced by the S.E.M. It would be a great mistake to drop this stepping-stone from all future illustrations of insect eggs. As "the field" *still* remains our source of all new material (and of inspiration, in many cases), the implications of the above should be abundantly apparent!

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